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The First Example of Lipase-catalyzed Resolution of a Stereogenic Center in Steroid Side Chains by Transesterification in Organic Solvent

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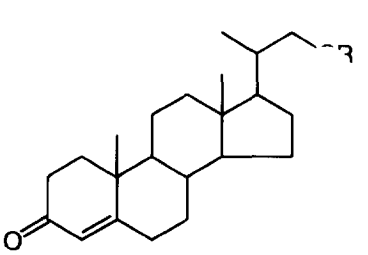
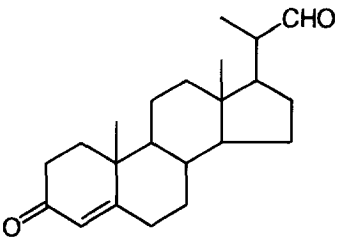
Abstract: The *Pseudomonas cepacia* lipase selectively catalyzes the acylation of the (20S)-isomer of the 22-alcohol group in the C-22 steroid compounds **1a** and **4a** when the transesterification is irreversibly carried out with vinyl acetate in an organic solvent. Copyright © 1996 Elsevier Science Ltd

The lipase-catalyzed transesterification in organic solvents of hydroxylated substrates is now a well established method extensively applied to the synthesis of enantiomerically pure compounds.[1] In principle, this reaction should be extremely useful for steroids that are highly insoluble in water and in fact the regioselective acylation and deacylation of a few steroid compounds has been already described by several authors[2] and recently reviewed.[3] Different lipases have been used in the reported studies and most of the hydroxy groups were located in the steroid rings. We wish to report our results on the stereoselective transesterification of a hydroxy group in the steroid side chain, using a *Pseudomonas cepacia* lipase[4] as biocatalyst in the conditions of irreversible transesterification in an organic solvent,[5] a method that we have used for the enantioselective resolution of a variety of 2-substituted alkanols.[6] We chose as steroid substrate the C-22 alcohol **1a** prepared as a mixture of 20R and 20S epimers by reduction of the corresponding 20S-aldehyde that easily epimerizes in the reaction conditions.[7]



1

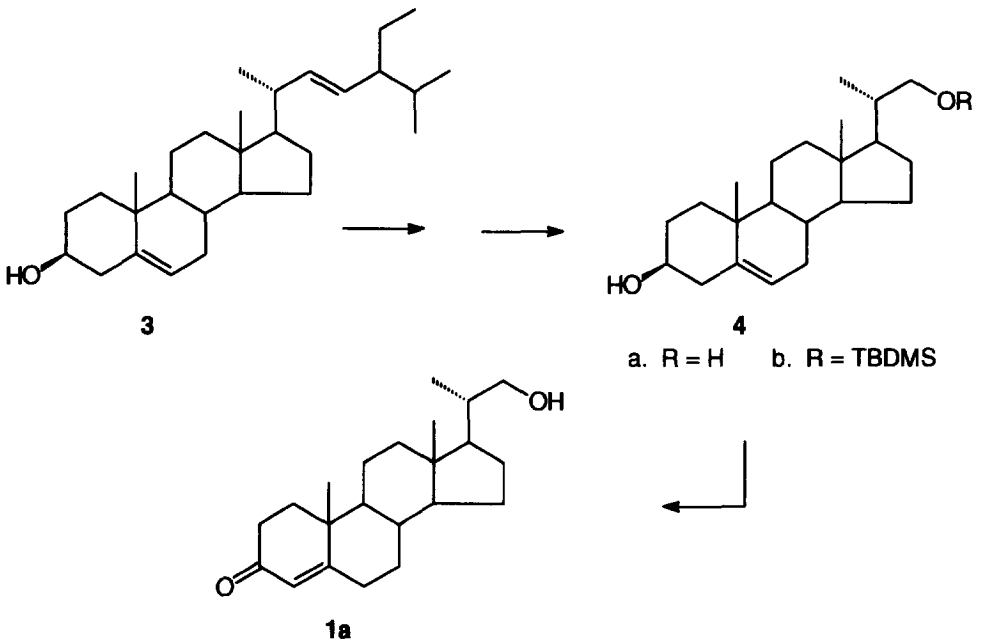
a. R = H b. R = TBDMS



2

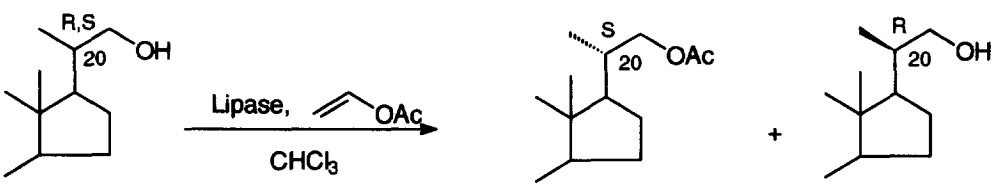
A chemoselective reduction of commercially available 4-pregnen-3-one 20β-carboxaldehyde **2** (Sigma, USA) to the required **1a** can be realized by $NaBH_4$/PEG 400[8] that was formed as a 2:1 isomeric ratio of 20S

and 20R-**1a**, as established by GLC analysis,[9] using as a reference 20S **1a** a sample prepared from stigmasterol **3** by the synthetic sequence depicted in the Scheme.[10]



Scheme

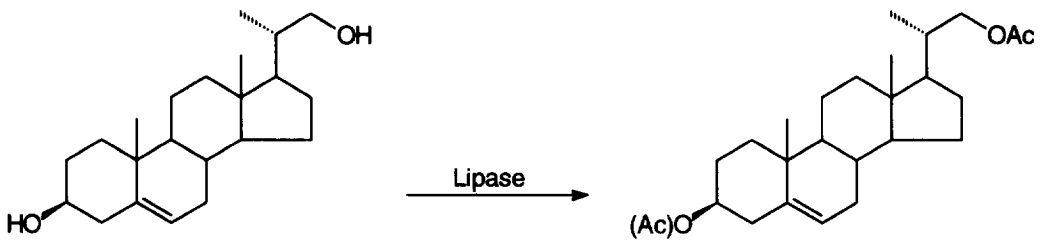
The 20 R,S-alcohol **1a** prepared as above was subjected to the reaction with lipase and vinyl acetate in chloroform[11] and after 68 h, 70% of the acetate **1c** was formed. GLC analysis of the unreacted alcohol **1a** showed that it consisted of a 20R/20S mixture, 4:1.[12] Apparently only 20 S **1a** had reacted and this was clearly proved by GLC and 500 MHz ^{1}H-NMR analyses of the acetate **1c** enzymatically formed.[13]



(20 R,S)-1a **(20 S)-1c** **(20 R)-1a**

When the 20 S-**1a** (prepared from stigmasterol **3**, as previously described) was subjected to the same reaction with lipase in 68 h a conversion of 86% into the acetate **1c** was reached. This observation is in agreement with the fact that 20 S-**1a** is the substrate accepted by the enzyme in the conditions of the

transesterification reaction. This configurational outcome is in agreement with previous observations made by our group on the stereochemical requirements of 2-methyl alkanols under the enzymatic process[14] and includes the side chain of **1a** in this class of primary alcohols.[15] The result can be also explained considering that, with respect to the 18β-methyl, in the (20S)-isomer the methyl of the stereogenic center is in a less hindered position than in (20R)-**1a**. An additional information on the steric requirements of the lipase from *Pseudomonas cepacia* has been obtained by the transesterification of the dihydroxy compound **4a**, in a mixture of chloroform/tetrahydrofuran (2:1) that was required to prepare a solution of the substrate. In these conditions, in 30 h a conversion to 30% of acetate was reached, the 21-acetate being selectively formed with respect to the 3β-counterpart.[16]



4a **5**

This result offers an additional example of the regioselective control of the enzymatic reaction on a polyfunctional steroid substrate and all the preliminary informations collected in the present work open a new possible approach to the stereoselective construction of asymmetric steroid side chains, including the access to unnatural (i.e. 20 S) steroids.

Acknowledgements. This work has been financially supported by Ministero dell'Università e della Ricerca Scientifica (MURST).

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3. Riva, S. in *Applied Biocatalysis*, Blanch, H. W.; Clark, D. S.. Eds, M. Dekker, New York **1991**, Vol. 1, 179-219.
4. The lipase was a generous gift from Amano (Japan) through its italian subsidiary. We thank Mr. I. Gaudio (Mitsubishi Italia, Milano) for his kind collaboration.
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7. Wicha, J.; Bal, K. *J. Chem. Soc., Perkin 1* **1978**, 1282 and references cited herein.
8. Santaniello, E.; Ferraboschi, P.; Fiecchi, A.; Grisenti, P.; Manzocchi, A. *Gazz. Chim. Ital.* **1987**, *117*, 701.
9. GLC analysis (Hewlett Packard, mod. 5890/II, HP-5 capillary column, T=300 °C) showed two peaks for the isomers at T_R 13.9 and 14.2 min in 34:66 ratio.
10. The C-20 configuration of a natural steroid such as stigmasterol **3** is essentially retained during the synthetic sequence outlined in the Scheme. The experimental procedure for the steps **3** ⇒ **4a** essentially follows the protocols of the reactions used for the synthesis of (25S)-26-hydroxycholesterol described by us in another work (see: Ferraboschi, P.; Fiecchi, A.; Grisenti, P.; Santaniello, E. *J. Chem. Soc., Perkin 1.* **1987**, 1749.). The diol **4a** (30 % from **3**) was selectively transformed into the 22-TBDMS ether **4b** (see: Ogilvie, K. K.; Schifman, A. L.; Penney, C. L. *Can. J. Chem.* **1979**, *57*, 2230) that was subsequently converted to **1b** (35% from **4a**) by an Oppenauer oxidation (*Organic Reactions in Steroid Chemistry*, Fried, J. and Edwards, J. A. Eds., van Nostrand Reinhold, New York, Vol.1, p. 234-237). The silyl protection was selectively cleaved ($LiBF_4$) according to Metcalf, B. W.; Jund, K.; Burkhart, J. P. *Tetrahedron Lett.* **1980**, *21*, 15) to 20S **1a** that in GLC corresponded to the most abundant isomer from **2** (66%, T_R 14.2 min).
11. A solution of 20 R,S-**1a** (0.33 g, 1 mmol) in chloroform (2.2 mL) and vinyl acetate (0.43 mL, 4.66 mmol) was added to the solid lipase (14 m g, 31.5 U/mg) under stirring at room temperarure.
12. The most significant resonances in the ^{1}H-NMR (500 MHz) of the unreacted **1a** were assigned as follows: 0.69 (s, 3 H, 18-CH_3), 0.91 (d, 2.4 H, 21-CH_3), 1.01 (d, 0.6 H, 21-CH_3), 1.15 (s, 3 H, 19-CH_3), 3.30-3.37 (m, 0.2 H, CH_2O), 3.37-3.45 (m, 0.8 H, CH_2O), 3.58-3.63 (m, 0.2 H, CH_2O), 3.65-3.72 (m, 0.8 H, CH_2O).
13. The enzymatically formed **1c** has T_R 16.3 min, identical to the acetate from synthetic 20S-**1a** and the ^{1}H-NMR (500 MHz) spectrum of the two samples were superimposable.The most significant resonances in the ^{1}H-NMR (500 MHz) of the 20S -**1c** were assigned as follows: 0.67 (s, 3 H, 18-CH_3), 0.94 (d, 3 H, 21-CH_3), 1.12 (s, 3 H, 19-CH_3), 2.00 (s, 3 H, CH_3CO), 3.70-3.80 (m, 1 H, CH_2O), 4.00-4.08 (m, 1 H, CH_2O).
14. Ferraboschi, P.; Casati, S.; De Grandi, S.; Grisenti, P.; Santaniello, E. *Biocatalysis* **1994**, *10*, 279.
15. A recent interpretation of the enantiopreference of *Pseudomonas cepacia* lipase toward primary alcohols has been proposed; see: Weissfloch, A. N. E.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 6959.
16. ^{1}H-NMR (500 MHz) of the enzymatic acetate **5**: 0.68 (s, 3 H, 18-CH_3), 0.97-1.01 (d+s, 6 H, 19 and 21-CH_3), 2.02 (s, 3 H, -CH_3CO), 3.42-3.54 (m, 1 H, CHO), 3.72-3.78 (m, 1 H, CH_2O), 4.02-4.08 (m, 1 H, CH_2O), 5.29-5.36 (m, 1 H, CH=).

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